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REMARKS

In order to expedite issuance of this application, claims 3-6, 9-14, and 16-57 are cancelled without prejudice.

Claims 1, 2, 15, and 58 are amended.

Claim 1 is amended to clarify that the first library recited in step (i) comprises polynucleotide clones, and the second library recited in step (ii) comprises proteins and polypeptides produced expression of the polynucleotides from the first library. Claim 1 is also amended to specify that the polynucleotides are expressed in step (ii) by *in vitro* transcription and translation as previously set forth in cancelled claim 11. Step (iv) of claim 1 is also amended to clarify that the protein or polypeptide selected in step (iii) is identified by sequencing a polynucleotide clone from the first library that encodes for the individual protein or polypeptide selected in step (iii). Support can be found on page 3, lines 8-24. No new matter is added by this amendment.

Claim 2 is amended to clarify the types of biological activity recited in the claim. Support for this amendment can be found in the specification at page 13, line 16 through page 14, line 30. No new matter is added by this amendment.

Claim 15 is amended to clarify that the first library recited in step (i) comprises polynucleotides, and the second library in step (ii) comprises proteins and polypeptides obtained by *in vitro* transcription and translation of the polynucleotides from the first library. Support can be found at page 3 lines 8-30 and page 9 line 28 through page 10, line 19. No new matter is added by this amendment.

Claim 58 is amended to clarify that the polynucleotides from bacteria in the first library are expressed by *in vitro* transcription and translation and to clarify that the biological activity is the ability of a protein or polypeptide in the second library to effect a post-translational modification of a protein or polypeptide from a tissue extract. Support can be found in the specification at page 19, line 28 through page 20, line 3. No new matter is added by this amendment.

Claims 1, 2, 15, and 58 were rejected as purportedly being indefinite. The currently amended claims are deemed to be clear and definite.. Accordingly, applicants

request that the rejections of claims 1, 2, 15 and 58 for indefiniteness be withdrawn.

Claims 1, 2, 7, 8, 15, 58, 60-62 and 64-65 have been rejected as purportedly being anticipated by Thompson *et al.* This rejection is unwarranted. All of these claims are presently directed to methods for screening proteins and polypeptides, which involve generating a first library comprising polynucleotides, and then generating a second library comprising proteins and polypeptides. The second library is generated by *in vitro* transcription and translation of the polynucleotides from the first library.

Thompson *et al.* deals exclusively with methods involving expression of proteins in living cells. Thompson *et al.* does not teach or suggest a screening method involving *in vitro* transcription and translation, which is a cell-free process utilizing purified enzymes and cell extracts, but not living cells (See e.g., attached abstract of F. Movahedzadeh, *In Vitro Transcription and Translation*, Chapter entitled "E. coli Plasmid Vectors Methods and Applications", pp. 247-456, ISBN 1-59259-409-3 (2003); and attached page 162 of the Novagen 2004/2005 Catalog, describing *in vitro* transcription and translation). The term "library clones" as used in Thompson *et al.* refers to libraries of transformed living host cells and organisms (see col. 33, ¶2). The recitations of the term "*in vitro*" found in Thompson *et al.*, which were cited on page 9 of the Office Action, relate to *in vitro* packaging of genes to introduce the genes into living cells (col. 21, ¶2; col. 28, ¶4), to *in vitro* recombination of genes in viruses to provide expression vectors for introduction into living host cells (col. 23, ¶3), and to the use of a library of living cells in an *in vitro* assay (col. 33, ¶1). None of the references to "*in vitro*" in Thompson *et al.* relates to the cell-free expression of proteins from polynucleotides by *in vitro* transcription and translation, as presently claimed. Accordingly, all of the present claims are clearly patentable over Thompson *et al.*

Claims 1, 2, 7, 15, 58-65 have been rejected as being anticipated by McCarthy *et al.* This rejection is also unwarranted. Contrary to the assertions in the Office Action, McCarthy *et al.* does not teach or suggest the formation of a *second library* comprising proteins and polypeptides. None of the steps of the method set forth in the abstract or the specification of McCarthy *et al.* describe forming a library of proteins and polypeptides from a library of polynucleotides. Step (f) in the method of McCarthy *et al.* states: ". . . identifying

a clone in the mammalian cell clone library which express alkaline phosphatase . . ." This phrase *does not* describe formation of a *library* of individual proteins and polypeptides from a library of polynucleotide clones. Furthermore, all of the present claims require that the second library (i.e., the protein and polypeptide library) be prepared by *in vitro* transcription and translation of the polynucleotides in the first library. McCarthy *et al.* only refers to *in vitro* assays for *isolating and purifying secreted proteins* at column 1, lines 19-21. An *in vitro* assay for isolating and purifying secreted proteins is not *in vitro* transcription and translation. "Secreted proteins" refers to proteins produced in living cells and secreted through the cell membrane into the culture medium. There is no teaching or suggestion in McCarthy *et al.* of a method of screening proteins and polypeptides by forming a first library comprising polynucleotides and then forming a second library of proteins and polypeptides from the first library by *in vitro* transcription and translation, as claimed by Applicants. Accordingly, all of the present claims are patentable over McCarthy *et al.*

Reconsideration and allowance of all claims is earnestly solicited. In the event that the foregoing is not deemed persuasive, Applicants request that the present amendment be entered to place the application in better form for appeal.

Respectfully submitted,

Dated 27 April 2004

By _____
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In Vitro Transcription and Translation

E. coli Plasmid Vectors

Methods and Applications
July 2003
pps. 247-256

ISBN:
Series: Methods in Molecular Biology
Volume #: 235

Farahnaz Movahedzadeh
Susana González Rico
Robert A. Cox

File size: 123 KB

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Abstract:

In this chapter, we describe the use of plasmid vectors in transcription and translationsystems in vitro to investigate aspects of the biology of the gene and the protein forwhich it codes. An in vitro, or cell-free, assay reproduces a relatively complex physiologicalprocess by mixing the essential purified components of the system under controlledconditions outside of the cell. Such systems allow the basic steps of transcriptionand translation to be studied individually, and the products obtained at each ste to bealtered in different ways according to the needs of the research . Thus, an in vitrosyste is convenient when it is necessary to modify a product, for example, by introducingmutation labels, tags, or fusions.

Keywords:
Language: English

Citations show citations

Single Tube Protein® System 3

Efficient transcription/translation in a single tube

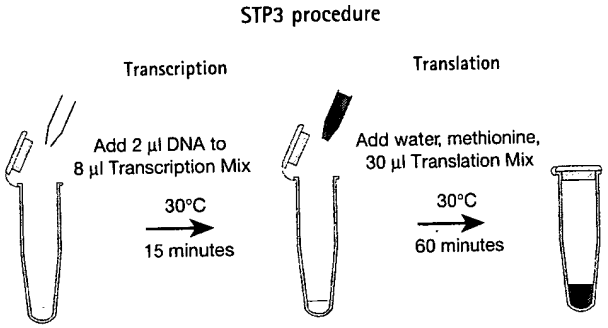
The Single Tube Protein® System 3 (STP3®) is designed for efficient *in vitro* production of proteins directly from supercoiled or linear DNA templates. The method is based on a linked reaction in which transcription by a bacteriophage RNA polymerase is directly followed by translation in an optimized rabbit reticulocyte lysate. Unlike other commercial kits, the patented STP3 method inherently provides an optimal amount of RNA template for translation, which results in superior protein yield and consistency between samples.

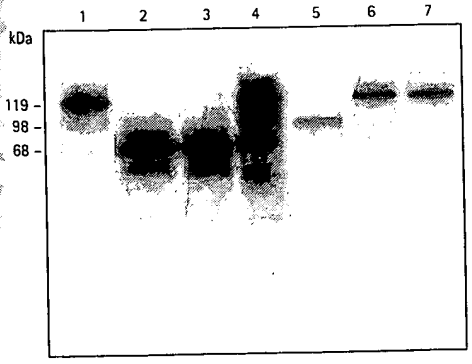
Any DNA coding sequence inserted downstream from a T7 or SP6 promoter can be quickly expressed as protein simply by adding it to the linked reaction. Suitable templates can be supercoiled or linearized plasmid DNA or PCR products. Compared with standard methods for translation of synthetic RNA templates, several tedious manipulations are avoided, including restriction enzyme digestion, linearized plasmid DNA purification, and RNA purification, which normally require four to six hours to perform. In addition, the STP3 method efficiently produces protein directly from PCR amplification products generated using appropriately designed primers (see next page for additional information). Performance can be further enhanced using the Novagen pCITE® or pT7Blue-2 vectors containing eukaryotic translation enhancer sequences.

In the standard STP3 reaction, the DNA template (typically 0.5 µg plasmid or 2 µl chloroform-extracted PCR amplification reaction) is transcribed at 30°C for 15 minutes, followed by the addition of translation mix and continued incubation for 60 minutes. All components are premixed such that the only reagents to be added are template, water, and a choice of unlabeled methionine (included) or ^{35}S-methionine (not included).

The standard kit has enough reagents to perform 50 standard 50-µl reactions or 100 small-scale (25-µl) reactions. An introductory kit for 10 standard reactions is also available. A positive control DNA containing the *E. coli* β-galactosidase gene is included with the kits to monitor performance. The translation product of the STP3 control reaction can be measured by standard incorporation assays, nonradioactive S•Tag™ Rapid Assay or FRETWorks™ S•Tag Assay, S•Tag™ Western Blot, or direct measurement of β-galactosidase activity with the BetaFluor™ β-Gal Assay Kit. Careful quality control ensures that the Single Tube Protein System 3 provides the highest activity, lowest background, and most consistent performance available.

continued on next page



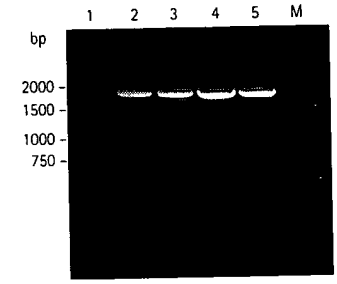


Lane	Construct	DNA Form
1	pCITE β-gal	plasmid
2	pBAC™ T7 GUS	plasmid
3	pT7Blue-2 GUS	PCR
4	pT7Blue-2 β-gal	PCR
5	pET-32 ORF86	PCR
6	pT7Blue-2 β-gal	plasmid
7	pSCREEN™ β-gal	plasmid

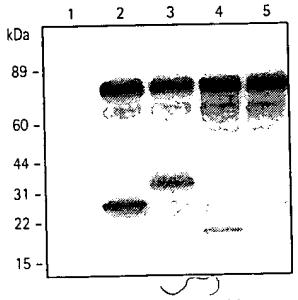
STP3 performance with various templates

The indicated templates were transcribed/translated under STP3 standard conditions in the presence of ^{35}S-methionine. Reaction products were analyzed by 4-20% SDS-PAGE and fluorography (gel exposed 2 h). For PCR templates, plasmids were amplified using primers upstream of the T7 promoter and downstream of the coding sequence. Completed amplification reactions were extracted with 1 volume of chloroform and 2 µl of the aqueous phase were added directly to the STP3 transcription reaction. Samples 1–6 were STP3, T7 reactions and sample 7 was an STP3, SP6 reaction.

A. PCR Products



B. STP3 Products



STP3 analysis of a genomic DNA exon

A region of exon 15 of the human APC gene was PCR-amplified from genomic DNA isolated from several individuals (expected PCR product size = 1818 bp). The 37-bp 5'-primer contained a T7 promoter and translation initiation sequence in-frame with the exon coding region plus a 6-bp upstream spacer sequence. After extraction with chloroform, the PCR products (1 µl of a 50-µl reaction) were directly added to a standard STP3 reaction. Panel A shows agarose gel analysis of the PCR products (5 µl samples) and Panel B shows the ^{35}S-labeled STP3 reaction products of the corresponding samples in Panel A analyzed by SDS-PAGE and fluorography. The gel was exposed for 16 hours. Lanes 2–4 were samples having known truncating mutations and were kindly provided by the Johns Hopkins Hereditary Colorectal Cancer Registry. Lane 1 contained no genomic DNA and lane 5 contained Human Genomic DNA (Cat. No. 69237-3).

Shipping and Storage Information: begins on page 410

Ordering and Bulk Order Information: see pages 16 and 17

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